Serum Hormones and the Alcohol–Breast Cancer Association in Postmenopausal Women

Joanne F. Dorgan, David J. Baer, Paul S. Albert, Joseph T. Judd, Ellen D. Brown, Donald K. Corle, William S. Campbell, Terryl J. Hartman, Aliya A. Tejpar, Beverly A. Clevidence, Carol A. Giffen, Donald W. Chandler, Frank Z. Stanczyk, Philip R. Taylor

Background: **Alcohol ingestion is associated with an increased risk of breast cancer in most epidemiologic studies. Results, however, are heterogeneous at lower levels of alcohol intake, and a biologic mechanism for the association has not been clearly identified. To determine whether alcohol consumption by postmenopausal women elevates serum levels of hormones associated with an increased risk of breast cancer, we performed a controlled feeding study.** ***Methods:*** **Participants were 51 healthy postmenopausal women not using hormone replacement therapy. Each participant rotated through three 8-week dietary periods in which she consumed 15 or 30 g of alcohol per day or an alcohol-free placebo beverage. The order of assignment to the three alcohol levels was random. During the dietary periods, all food and beverages were supplied by the study, and energy intake was adjusted to keep body weight constant. Levels of estradiol, estrone, estrone sulfate, testosterone, androstenedione, progesterone, dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), and androstenediol were measured by radioimmunoassays in serum collected at the end of each dietary period. All statistical tests are two-sided.** ***Results:*** **When women consumed 15 or 30 g of alcohol per day, respectively, estrone sulfate concentrations increased by 7.5% (95% confidence interval [CI] = −0.3% to 15.9%; *P* = .06) and 10.7% (95% CI = 2.7% to 19.3%; *P* = .009) and DHEAS concentrations increased by 5.1% (95% CI = 1.4% to 9.0%; *P* = .008) and 7.5% (95% CI = 3.7% to 11.5%; *P*<.001) relative to levels when women consumed placebo. None of the other hormones measured changed statistically significantly when women consumed alcohol.** ***Conclusions:*** **Results suggest a possible mechanism by which consumption of one or two alcoholic drinks per day by postmenopausal women could increase their risk of breast cancer. [J Natl Cancer Inst 2001;93: 710–5]**

The relationship between ingestion of alcohol and the risk of breast cancer has been evaluated in numerous case–control and cohort studies [reviewed in *(1,2)*]. Most studies report positive associations, but results are heterogeneous, particularly at lower levels of alcohol intake. In a combined analysis of 38 studies *(1)*, consumption of one alcoholic drink per day was associated with a statistically significant 11% increased risk of breast cancer, and the risk increased with increasing intake (P_{trend} <.001). Similarly, in a pooled analysis of data from six cohort studies *(3),* each 10-g increment of alcohol intake per day statistically significantly increased breast cancer risk by 9%. The association of alcohol consumption with breast cancer risk does not vary by whether alcohol is consumed as wine, beer, or liquor *(1–3).*

Results of a recent meta-analysis *(4)* indicate that postmenopausal women who develop breast cancer have a mean serum estradiol concentration that is 15% higher than that in unaffected postmenopausal women (*P*<.001). Although results are less consistent for estrone and estrone sulfate, in an analysis from the Nurses' Health Study, postmenopausal women with elevated serum concentrations of these hormones had an approximately twofold excess risk of breast cancer *(5).* Serum concentrations of testosterone *(5–10),* androstenedione *(6,10),* dehydroepiandrosterone (DHEA) *(11),* DHEA sulfate (DHEAS) *(5,6,10,12),* and androstenediol *(11)* also have been reported to be statistically significantly higher in postmenopausal women who subsequently develop breast cancer than in unaffected postmenopausal women.

Clear evidence that alcohol consumption increases levels of hormones related to breast cancer would suggest a mechanism by which alcohol could increase breast cancer risk, thereby providing support for a causal relationship. Although some observational data show an association of alcohol ingestion with serum estrogen and androgen levels in postmenopausal women, results are inconsistent *(13–18).* In a metabolic study *(19),* ingestion of alcohol acutely raised serum estrone levels in postmenopausal women using hormone replacement therapy (HRT), but it did not affect serum estrogen levels in women not using HRT.

To clarify this issue, we performed a controlled feeding study. Our primary objective was to evaluate the effect of chronic moderate alcohol ingestion on serum and urine hormone levels in postmenopausal women not using HRT. Our secondary objective was to evaluate the effects of alcohol ingestion on serum lipids, micronutrients, oxidative stress, and DNA repair. We present results for serum hormones in this report. Our other results will be reported separately.

Subjects and Methods

Participants

The Women's Alcohol Study was conducted at the Beltsville Human Nutrition Research Center, U.S. Department of Agriculture, Beltsville, MD, from 1998 through 1999. The participants were recruited by posters and advertisements from communities around Beltsville. To be eligible, women had to meet the following requirements: 1) aged 50 years or older and postmenopausal (last menses at least 1 year earlier); 2) not using HRT; 3) being a nonsmoker; 4) having 90%–140% of ideal weight for height *(20);* 5) having at least one intact ovary; 6) having no major health problems, such as heart disease, stroke, diabetes, or cancer (other than nonmelanoma skin cancer); 7) not taking prescription medications that could interfere with the study; 8) having no food allergies; 9) willing to eat all the foods and only the foods supplied by the study; 10) having no history of alcohol abuse but not an ab-

Affiliations of authors: J. F. Dorgan, Fox Chase Cancer Center, Philadelphia, PA; D. J. Baer, J. T. Judd, E. D. Brown, B. A. Clevidence, Beltsville Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD; P. S. Albert (Division of Cancer Treatment), D. K. Corle (Division of Cancer Prevention), W. S. Campbell, A. A. Tejpar, P. R. Taylor (Division of Clinical Science), National Cancer Institute, Bethesda, MD; T. J. Hartman, Department of Nutrition, Pennsylvania State University, University Park; C. A. Giffen, Information Management Services, Inc., Silver Spring, MD; D. W. Chandler, Esoterix Endocrinology, Inc., Calabasas Hills, CA; F. Z. Stanczyk, Department of Obstetrics and Gynecology, University of Southern California School of Medicine, Los Angeles.

Correspondence to: Joanne F. Dorgan, M.P.H., Ph.D., Fox Chase Cancer Center, 7701 Burholme Ave., Philadelphia, PA 19111 (e-mail: JF_Dorgan@fccc.edu).

See "Notes" following "References."

stainer; and 11) having no history of alcoholism in their parents. The study was approved by institutional review boards at the National Cancer Institute (Bethesda, MD) and The Johns Hopkins University School of Hygiene and Public Health (Baltimore, MD). Before entering the study, the participants signed an informed consent form.

A total of 65 women completed baseline visits and were enrolled in the Women's Alcohol Study. Of these 65 women, 63 began the controlled feeding study and 53 completed the study. Eight women dropped out during the first dietary period, and two women dropped out during the washout period that followed. Two women found to be using glucocorticoids at the time of blood collection after consumption of the placebo beverage were subsequently excluded, leaving a total of 51 participants for analysis.

Experimental Design

The Women's Alcohol Study used a three-period crossover design. Each participant rotated through three 8-week controlled dietary periods when she consumed a different amount of alcohol—15 or 30 g of alcohol per day or an alcohol-free placebo beverage. The order of assignment to the three alcohol levels was random. Each dietary period was preceded by a 2- to 5-week washout period when women consumed no alcohol. All food and beverages, including alcoholic beverages, were prepared and supplied by the Beltsville Human Nutrition Research Center's Human Study Facility during the controlled feeding periods, and the participants were required to eat all items. Alcohol was supplied as 95% ethanol (Everclear™; Pharmco Products, Inc., Brookfield, CN) in orange juice (12 ounces). To replace energy from 30 g of alcohol, the diets with 0 or 15 g of alcohol were supplemented with energy from carbohydrates (Polycose™; Abbott Laboratories, Columbus, OH) and soft drinks. The participants were instructed to consume their study beverages with the snack supplied by the study over a period of 1–2 hours before bedtime after completing activities that require substantial manual dexterity such as driving an automobile. The participants were not told the alcohol content of the beverage.

Meals were prepared at the Beltsville Human Nutrition Research Center from typical U.S. foods on a 7-day menu cycle. Each day's diet provided 15% energy as protein, 50% energy as carbohydrate, and 35% energy as fat, with a polyunsaturated/monounsaturated/saturated fat ratio of 0.6 : 1 : 1. Daily dietary fiber intake was 10 g/1000 kcal, and daily cholesterol intake was 150 mg/1000 kcal. Diets provided 100% of the U.S. recommended dietary allowances for vitamins and minerals *(21);* with the exception of calcium and iron, supplements were prohibited. Each weekday, the participants were weighed and caloric intake was adjusted in 200-kcal increments as needed to maintain body weight constant throughout the study. On weekdays, the participants ate breakfast and supper at the center's dining facility, and a carryout lunch was provided. Weekend food and beverages were packaged for consumption at home.

Blood for hormone analyses was collected after an overnight fast between 6:30 AM and 9:00 AM on 3 days during the last week of each dietary period. Serum was separated, and aliquots were frozen at −70 °C. An equal volume of serum from each day was pooled for each dietary period for analysis of hormones and sex hormone-binding globulin (SHBG).

Laboratory Methods

Levels of DHEA and androstenediol in serum were measured at the Reproductive Endocrine Research Laboratory, University of Southern California School of Medicine, Los Angeles. The levels of all of the other hormones were measured by Esoterix Endocrinology, Inc. (Calabasas Hills, CA) using standard procedures. DHEA and androstenediol were extracted with hexane/ethyl acetate, 3 : 2 (vol/vol), and were then chromatographed on Celite (Celite Corp., Lompoc, CA) impregnated with ethylene glycol before quantification by radioimmunoassay (RIA). Elution of DHEA was carried out with 25% toluene in isooctane, and elution of androstenediol was carried out with 100% toluene *(11).* Levels of estradiol and estrone were measured by use of a modification of the procedure developed by Wu and Lundy *(22).* Serum samples were extracted with hexane/ethyl acetate, 80 : 20 (vol/vol). The extract was then washed with dilute base, concentrated, and chromatographed on Sephadex LH20 microcolumns (Sigma Chemical Co., St. Louis, MO). Estradiol and estrone were specifically eluted with benzene/methanol, 85 : 15 (vol/vol), and measured by RIA. Androstenedione was first extracted from serum with hexane/ethyl acetate, 99 : 1 (vol/vol). The extract was then separated from the aqueous phase by centrifugation (2200*g* for 2 minutes at room temperature), and aliquots were evaporated to dryness before quantification by RIA. The level of testosterone was measured by a modification of the procedure developed by Furuyama et al. *(23).* Samples were extracted with hexane/ethyl acetate, 90 : 10 (vol/vol), and the extract was applied to aluminum oxide micro-columns. The columns were washed with hexane containing 0.55% ethanol, and testosterone was specifically eluted with hexane containing 1.4% ethanol and quantified by RIA. Progesterone was extracted with hexane/ethyl acetate, 99 : 1 (vol/vol), and separated from the aqueous phase by centrifugation (2200*g* for 2 minutes at room temperature). Extracts were evaporated to dryness before quantification by RIA. DHEAS was measured by RIA as DHEA after enzymolysis of the DHEAS. SHBG was measured with an immunoradiometric assay. The serum sample and an SHBG monoclonal antibody labeled with ^{125}I were incubated with plastic beads coated with a different SHBG monoclonal antibody. The beads were washed to remove unbound label, and the bound radioactivity was measured. The percent non-SHBG-bound estradiol and the percent non-SHBG-bound testosterone were determined by ammonium sulfate precipitation as described previously by Nankin et al. *(24).* The concentration of non-SHBG-bound steroid was then calculated as the product of the percent non-SHBG-bound steroid and total concentration.

Samples from each participant were grouped in random order and were analyzed together in the same batch. Within-batch coefficients of variation estimated from hormone measurements on two to four masked quality-control samples included in each batch were as follows: estradiol = 12.1%, estrone = 16.8%, estrone sulfate = 7.8%, testosterone (one outlier excluded) = 11.0%, androstenedione = 6.3%, DHEA = 10.7%, DHEAS = 4.9%, progesterone = 20.7%, androstenediol = 9.4%, and SHBG = 3.1%.

Statistical Methods

All hormone concentrations were transformed to the $\log_e$ before statistical analyses, so that treatment effects could be evaluated as relative changes and error terms would be approximately normal. Changes in hormone concentrations from placebo on 15 and 30 g of alcohol per day were estimated by use of linear mixed models, including the participant as a random effect (i.e., a single random intercept) and alcohol levels as fixed effects treated as two indicator variables and, in separate models to test for trend, as a continuous variable with values 0, 15, and 30 *(25).* To assess the effect of model assumptions on study results, we also evaluated by paired Student's *t* tests the statistical significance of changes in hormone levels when 15 or 30 g of alcohol was consumed per day. These results were similar to those obtained from linear mixed models for all hormones (data not shown). Differences in hormone concentrations among the three dietary periods were evaluated by likelihood ratio tests of improvement in the model fit after addition of two indicator variables as fixed effects to models that included the participant as a random effect. Age, years since menopause, race, and baseline body mass index (BMI = weight in kg/[height in m]^2) were constant throughout the study for each participant and, therefore, could not confound associations between alcohol consumption and hormone levels. However, inclusion of characteristics associated with hormone levels in models could potentially improve the precision of parameter estimates for alcohol. Standard errors (not shown) of alcohol parameter estimates from simple models and from models that included characteristics statistically significantly associated with each hormone were compared to evaluate the effect of adjustment on precision. Effect modification by assignment order, dietary period, age, BMI, race, and years since menopause was assessed by likelihood ratio tests of improvement in a model fit after addition of cross-product terms to models that included main effects for alcohol and the characteristic being evaluated. Age, BMI, and years since menopause were modeled as continuous fixed effects. Assignment order, dietary period, and race were included as indicator variables. Because only two participants were Asian, tests for effect modification by race were restricted to whites and blacks. All tests of statistical significance were two-sided. All analyses were performed with S-PLUS *(26)* and SAS statistical analysis packages *(27).*

Results

The characteristics of the participants at baseline are summarized in Table 1. All participants were postmenopausal. Their ages ranged from 49.2 years to 78.8 years, with a median of 58.2 years. Most (74.5%) participants were white, 21.6% were black, and 3.9% were Asian. The participants reported consuming an average of 0.9 alcoholic beverage per week (range = 0–15 drinks per week) before

Table 1. Characteristics of participants at baseline (n = 51)

Characteristic	Median	Range
Age, y	58.2	49.2–78.8
Height, cm	163.1	152.1–179.7
Weight, kg	73.2	42.1–117.4
Body mass index, kg/m^2	26.9	17.7–42.5
Years since last menses*	9	0.75–35
Parity, No. of children	2	0–8

Characteristic	No.	%
Race		
White	38	74.5
Black	11	21.6
Asian	2	3.9
Educational level, y†		
<12	5	10.0
12	17	34.0
13–15	12	24.0
16	7	14.0
>16	9	18.0
Parous		
Yes	43	84.3
No	8	15.7
Menopause type		
Natural	42	82.4
Hysterectomy	9	17.6

*One participant who was 9 months since last menses at the start of the first washout period had a baseline serum follicle-stimulating hormone concentration of 101 mIU/mL. All other participants were at least 1 year since last menses when they began the study.

†One missing value.

the study. The average body weights (± standard deviation) were 73.4 kg (±17.0 kg), 73.1 kg (±16.3 kg), and 73.1 kg (±16.8 kg) when participants consumed no alcohol, 15 g of alcohol per day, or 30 g of alcohol per day, respectively.

The mean hormone concentrations in the participants when not consuming alcohol and the percent changes from no alcohol consumption when consuming 15 or 30 g of alcohol per day are summarized in Table 2. Because inclusion of age, years since menopause, race, and baseline BMI in models did not change the precision of parameter estimates for alcohol, results from simple models are presented. Estrone sulfate was the only estrogen that statistically significantly changed concentrations when women consumed alcohol; it increased by an average of 7.5% ($P = .06$) when women consumed 15 g of alcohol per day and by 10.7% ($P = .009$) when women consumed 30 g of alcohol per day. DHEAS concentrations also increased statistically significantly by an average of 5.1% ($P = .008$) and 7.5% ($P<.001$) when women consumed 15 or 30 g of alcohol per day, respectively. Trend tests for both estrone sulfate ($P = .009$) and DHEAS ($P<.001$) were highly statistically significant. None of the other hormones measured changed statistically significantly when women consumed alcohol.

The effect of alcohol on serum levels of hormones and SHBG did not vary with age, BMI, race, or years since menopause. None of the hormone concentrations differed among dietary periods, and the order of assignment to no alcohol or 15 or 30 g of alcohol per day did not modify associations with any of the hormones or SHBG.

Discussion

Alcohol ingestion statistically significantly increased postmenopausal women's serum estrone sulfate and DHEAS concentrations in this controlled feeding study. After the women consumed for 8 weeks 15 or 30 g of alcohol per day, respectively, estrone sulfate concentrations increased by 7.5% and 10.7% and DHEAS concentrations increased by 5.1% and 7.5%. Postmenopausal women with elevated serum estrone sulfate *(5,10)* and DHEAS *(5,6,10,12)* levels were reported to be at an increased risk of breast cancer in several recent prospective cohort studies. Results from our study showing that ingestion of the alcohol equivalent of one or two drinks per day statistically significantly raises serum levels of these hormones provide a mechanism by which moderate alcohol ingestion could increase breast cancer risk in postmenopausal women.

To our knowledge, this is the first study to evaluate, under controlled conditions, the effects of chronic moderate alcohol ingestion on levels of serum estrogens and androgens in postmenopausal women. The increase in serum estrone sulfate levels that we observed after alcohol ingestion is consistent with results of a cross-sectional analysis from the Nurses' Health Study in which the serum levels of estrone sulfate in postmenopausal women were positively correlated with their reported usual alcohol ingestion ($r = .17$; $P = .02$) *(13).* The increase in serum DHEAS concentration that we observed in this study of postmenopausal women after alcohol ingestion is consistent with our previously reported results for premenopausal women *(28).* In our earlier study *(28),* consumption of 30 g of alcohol per day increased plasma DHEAS levels by approximately 7% during both the follicular and the luteal phases of the menstrual cycle. DHEAS concentration in

Table 2. Geometric mean of serum hormone levels in participants when not consuming alcohol and percent change (Δ) in hormone levels from no alcohol consumption to 15 g and 30 g of alcohol consumed per day

Hormone	No alcohol, mean (95% CI)	15 g alcohol per day, Δ (95% CI)*	30 g alcohol per day, Δ (95% CI)*	P_{trend}†
Estradiol	0.69 (0.55 to 0.87) ngdL	−7.7% (−22.4% to 9.7%)	5.3% (−11.4% to 25.2%)	.56
Non-sex hormone-binding globulin (SHBG)-bound estradiol	0.34 (0.26 to 0.44) ng/dL	−2.4% (−16.2% to 13.7%)	8.9% (−6.5% to 26.9%)	.27
Estrone	2.9 (2.5 to 3.3) ng/dL	−1.6% (−9.1% to 6.6%)	1.8% (−6.0% to 10.3%)	.66
Estrone sulfate	47.1 (40.7 to 54.6) ng/dL	7.5% (−0.3% to 15.9%)	10.7% (2.7% to 19.3%)	.009
Testosterone	17.2 (14.6 to 20.3) ng/dL	0.8% (−5.6% to 7.7%)	1.1% (−5.3% to 8.0%)	.74
Non-SHBG-bound testosterone	3.5 (2.9 to 4.3) ng/dL	4.2% (−2.2% to 11.1%)	2.5% (−3.8% to 9.3%)	.45
Androstenedione	81.4 (74.8 to 88.6) ng/dL	−1.5% (−6.1% to 3.2%)	−1.4% (−6.0% to 3.3%)	.55
Dehydroepiandrosterone (DHEA)	295 (252 to 347) ng/dL	−3.2% (−10.1% to 4.2%)	−1.8% (−8.7% to 5.7%)	.63
DHEA sulfate	54.9 (46.0 to 65.4) μg/dL	5.1% (1.4% to 9.0%)	7.5% (3.7% to 11.5%)	<.001
Progesterone	15.7 (13.5 to 18.3) ng/dL	−11.1% (−22.4% to 1.8%)	−5.0% (−17.0% to 8.8%)	.47
Androstenediol	43.7 (38.3 to 49.8) ng/dL	4.4% (−1.3% to 10.4%)	4.9% (−0.8% to 11.0%)	.09
SHBG	76.0 (65.3 to 88.5) nmol/L	−3.7% (−7.9% to 0.7%)	−1.1% (−5.4% to 3.4%)	.64

*Estimates of percent change are from linear mixed models, including participant as a random effect and alcohol levels as fixed effects treated as two indicator variables.

†P_{trend} values (two-sided) are from linear mixed models, including participant as a random effect and alcohol level as a continuous fixed effect with values 0, 15, and 30.

postmenopausal women has also been positively related to alcohol ingestion in cross-sectional analyses *(16),* but results are not totally consistent *(17).*

Ginsberg et al. *(19)* evaluated the acute effects of high-dose alcohol ingestion (0.7 g/kg of body weight in 15 minutes) on serum estradiol and estrone levels in postmenopausal women under controlled conditions. For 5 hours after alcohol ingestion, the serum estrone concentration was statistically significantly elevated in women using HRT, but the serum concentrations of estradiol and estrone were unchanged in women not using HRT. None of the women in our study were using HRT, and the lack of an effect of chronic moderate alcohol ingestion on serum estradiol and estrone levels in our study is consistent with results of the metabolic study by Ginsberg et al. *(19)* and results of several cross-sectional studies conducted in perimenopausal *(29)* and postmenopausal *(13,17,18)* women. However, statistically significant gradients of increasing serum estradiol concentrations with increasing alcohol ingestion among postmenopausal women have also been reported *(14–16).*

Few studies have evaluated the relationship between alcohol ingestion and serum androgen levels in postmenopausal women. Similar to our findings, postmenopausal women's serum androstenedione and testosterone levels were not associated with alcohol ingestion in three cross-sectional studies *(15,17,19).*

DHEAS is exclusively secreted by the adrenal glands *(30),* and elevated serum levels of DHEAS after alcohol ingestion suggest stimulation of adrenal steroidogenesis. Although studies in humans are conflicting *(31–36),* alcohol consistently stimulates the hypothalamic–pituitary–adrenal axis in animals *(37).* The adrenal glands also secrete androstenedione and DHEA. Although we did not observe effects of alcohol ingestion on serum levels of these hormones, androstenedione and DHEA are also secreted by the ovaries and produced peripherally *(30).* Therefore, changes in adrenal production could have been masked by changes in production elsewhere. Androstenedione and DHEA have serum half-lives of only about 1 hour, whereas DHEAS has a serum half-life of almost 14 hours *(38,39).* Because we collected blood for hormone analyses approximately 10 hours after women consumed alcohol, we would have missed any transient elevations in serum levels of androstenedione and DHEA that may have occurred.

After menopause, estrogens are formed from androgens in peripheral tissues by aromatase *(40).* Increased production of precursor androgens by the adrenal glands could account for the elevation in estrone sulfate levels that we observed in women after alcohol ingestion. Although we did not observe an effect of alcohol on estrone levels, most of the estrone formed in peripheral tissues is converted to estrone sulfate by the estrone sulfotransferase in these tissues and the liver *(41).* Furthermore, the serum half-life is only 35 minutes for estrone compared with 5–7 hours for estrone sulfate *(42,43).* We measured estrone sulfate and DHEAS because they are, respectively, the most abundant circulating estrogen and steroid hormone. We possibly would have found the levels of the sulfated forms of other steroid hormones (e.g., estradiol sulfate) also to be elevated in serum of women after alcohol ingestion if we had measured them.

Estrogen sulfotransferase and hydroxysteroid sulfotransferase are the sulfotransferases principally responsible for sulfating estrone and DHEA, respectively. As part of its stimulatory effect on the adrenal glands, corticotropin stimulates adrenal hydroxysteroid sulfotransferase *(44);* therefore, alcohol would be expected to increase sulfation of DHEA in the adrenal glands through its purported effect on the hypothalamic–pituitary–adrenal axis. Alcoholic cirrhosis diminishes hydroxysteroid sulfotransferase activity in the liver, but this effect is the result of reduced enzyme content caused by chronic liver disease rather than by alcohol *per se (45).* We are not aware of any studies that have evaluated the effect of moderate alcohol ingestion on estrogen and hydroxysteroid sulfotransferase activity.

In our study, ingestion of the alcohol equivalent of one or two drinks per day did not affect the serum levels of estradiol, which is the most potent naturally occurring estrogen and is believed to play a key role in breast cancer etiology *(46).* However, serum levels of estradiol are low in postmenopausal women and were frequently below or close to the limit of detection of the assay in our participants. Furthermore, because estradiol is cleared rapidly from the circulation *(42),* we would have missed transient elevations, which may have occurred immediately after alcohol ingestion. Even if alcohol did not increase serum levels of estradiol, it could increase estradiol levels in the breast. Both normal and neoplastic breast tissues contain the enzymes necessary to metabolize androgens and estrone sulfate to estradiol *(47,48).* The relative contribution of these precursors to the formation of estradiol in the breast is controversial; however, in a recent study *(49),* the activity of breast tumor sulfatase exceeded that of aromatase by a factor of 10–100.

Elevated intracellular estrogens could act through the estrogen receptor to promote breast tumor growth. Although the association of alcohol consumption with breast cancer risk is stronger for estrogen receptor-positive tumors in some studies *(50,51),* in other studies *(52–55),* the association is stronger for estrogen receptor-negative tumors or does not differ by receptor status. Stimulation of local synthesis of growth factors *(56,57)* and polyamines *(58)* and formation of DNA adducts as a consequence of 4-hydroxy-estrogen metabolism *(59,60)* are alternative mechanisms by which estrogens could affect breast cancer risk that do not involve the estrogen receptor.

We evaluated the effect of alcohol ingestion on serum levels of 11 hormones and SHBG. After application of the Bonferroni correction for multiple comparisons *(61),* P_{trend} values for estrone sulfate and DHEAS were .108 and .001, respectively. Therefore, although it is unlikely that our DHEAS results were due to chance, a role for chance in our estrone sulfate results cannot be ruled out. However, given the strength of the estrone sulfate association, its biologic plausibility, and its consistency with the literature, we believe that it is unlikely to have been due to chance.

In summary, ingestion of one or two alcoholic drinks per day increased the levels of serum estrone sulfate and DHEAS in postmenopausal women. These hormones have been associated with an increased risk of breast cancer in prospective studies. Our results, therefore, suggest a mechanism by which moderate alcohol ingestion could modify breast cancer risk in postmenopausal women and provide support for a causal association. Alcohol has numerous physiologic effects and could also influence breast cancer risk through nonhormonal mechanisms.

REFERENCES

(1) Longnecker MP. Alcoholic beverage consumption in relation to risk of breast cancer: meta-analysis and review. Cancer Causes Control 1994;5:73–82.

(2) Rosenberg L, Metzger LS, Palmer JR. Alcohol consumption and risk of breast cancer: a review of the epidemiologic evidence. Epidemiol Rev 1993;15:133–44.

(3) Smith-Warner SA, Spiegelman D, Yaun SS, van den Brandt PA, Folsom AR, Goldbohm RA, et al. Alcohol and breast cancer in women: a pooled analysis of cohort studies. JAMA 1998;279:535–40.

(4) Thomas HV, Reeves GK, Key TJ. Endogenous estrogen and postmenopausal breast cancer: a quantitative review. Cancer Causes Control 1997;8:922–8.

(5) Hankinson SE, Willett WC, Manson JE, Colditz GA, Hunter DJ, Spiegelman D, et al. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 1998;90:1292–9.

(6) Dorgan JF, Longcope C, Stephenson HE Jr, Falk RT, Miller R, Franz C, et al. Relation of prediagnostic serum estrogen and androgen levels to breast cancer risk. Cancer Epidemiol Biomarkers Prev 1996;5:533–9.

(7) Berrino F, Muti P, Micheli A, Bolelli G, Krogh V, Sciajno R, et al. Serum sex hormone levels after menopause and subsequent breast cancer. J Natl Cancer Inst 1996;88:291–6.

(8) Zeleniuch-Jacquotte A, Bruning PF, Bonfrer JM, Koenig KL, Shore RE, Kim MY, et al. Relation of serum levels of testosterone and dehydroepiandrosterone sulfate to risk of breast cancer in postmenopausal women. Am J Epidemiol 1997;145:1030–8.

(9) Thomas HV, Key TJ, Allen DS, Moore JW, Dowsett M, Fentiman IS, et al. A prospective study of endogenous serum hormone concentrations and breast cancer risk in postmenopausal women on the island of Guernsey. Br J Cancer 1997;76:401–5.

(10) Cauley JA, Lucas FL, Kuller LH, Stone K, Browner W, Cummings SR. Elevated serum estradiol and testosterone concentrations are associated with a high risk for breast cancer. Study of Osteoporotic Fractures Research Group. Ann Intern Med 1999;130:270–7.

(11) Dorgan JF, Stanczyk FZ, Longcope C, Stephenson HE Jr, Chang L, Miller R, et al. Relationship of serum dehydroepiandrosterone (DHEA), DHEA sulfate, and 5-androstene-3β,17β-diol to risk of breast cancer in postmenopausal women. Cancer Epidemiol Biomarkers Prev 1997;6:177–81.

(12) Gordon GB, Bush TL, Helzlsouer KJ, Miller SR, Comstock GW. Relationship of serum levels of dehydroepiandrosterone and dehydroepiandrosterone sulfate to risk of developing postmenopausal breast cancer. Cancer Res 1990;50:3859–62.

(13) Hankinson SE, Willett WC, Manson JE, Hunter DJ, Colditz GA, Stampfer MJ, et al. Alcohol, height, and adiposity in relation to estrogen and prolactin levels in postmenopausal women. J Natl Cancer Inst 1995;87:1297–302.

(14) Gavaler JS, Love K, Van Thiel D, Farholt S, Gluud C, Monteiro E, et al. An international study of the relationship between alcohol consumption and postmenopausal estradiol levels. Alcohol Alcohol Suppl 1991;1:327–30.

(15) Gavaler JS, Van Thiel DH. The association between moderate alcoholic beverage consumption and serum estradiol and testosterone levels in normal postmenopausal women: relationship to the literature. Alcohol Clin Exp Res 1992;16:87–92.

(16) Nagata C, Kabuto M, Takatsuka N, Shimizu H. Associations of alcohol, height, and reproductive factors with serum hormone concentrations in postmenopausal Japanese women. Steroid hormones in Japanese postmenopausal women. Breast Cancer Res Treat 1997;44:235–41.

(17) Newcomb PA, Klein R, Klein BE, Haffner S, Mares-Perlman J, Cruickshanks KJ, et al. Association of dietary and life-style factors with sex hormones in postmenopausal women. Epidemiology 1995;6:318–21.

(18) Cauley JA, Gutai JP, Kuller LH, LeDonne D, Powell JG. The epidemiology of serum sex hormones in postmenopausal women. Am J Epidemiol 1989;129:1120–31.

(19) Ginsburg ES, Mello NK, Mendelson JH, Barbieri RL, Teoh SK, Rothman M, et al. Effects of alcohol ingestion on estrogens in postmenopausal women. JAMA 1996;276:1747–51.

(20) Society of Actuaries and Association of Life Insurance Medical Directors of America. Weight of insured persons in the United States associated with lowest mortality. Philadelphia (PA): Association of Life Insurance Medical Directors of America; 1980.

(21) National Research Council Food and Nutrition Board. Recommended dietary allowances. 10th ed. Washington (DC): National Academy Press; 1989.

(22) Wu CH, Lundy LE. Radioimmunoassay of plasma estrogens. Steroids 1971;18:91–111.

(23) Furuyama S, Mayes DM, Nugent CA. A radioimmunoassay for plasma testosterone. Steroids 1970;16:415–28.

(24) Nankin HR, Pinto R, Fan DF, Troen P. Daytime titers of testosterone, LH, estrone, estradiol, and testosterone-binding protein: acute effects of LH and LH-releasing hormone in men. J Clin Endocrinol Metab 1975;41:271–81.

(25) Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics 1982;38:963–74.

(26) MathSoft Data Analysis Products Division. S-PLUS 2000. Seattle (WA): MathSoft; 1999.

(27) SAS Institute, Inc. SAS/STAT user's guide. Version 8. Cary (NC): SAS Institute, Inc.; 1999.

(28) Reichman ME, Judd JT, Longcope C, Schatzkin A, Clevidence BA, Nair PP, et al. Effects of alcohol consumption on plasma and urinary hormone concentrations in premenopausal women. J Natl Cancer Inst 1993;85:722–7.

(29) London S, Willett W, Longcope C, McKinlay S. Alcohol and other dietary factors in relation to serum hormone concentrations in women at climacteric. Am J Clin Nutr 1991;53:166–71.

(30) Longcope C. Adrenal and gonadal androgen secretion in normal females. Clin Endocrinol Metab 1986;15:213–28.

(31) Schuckit MA, Gold E, Risch C. Plasma cortisol levels following ethanol in sons of alcoholics and controls. Arch Gen Psychiatry 1987;44:942–5.

(32) Lex BW, Ellingboe JE, Teoh SK, Mendelson JH, Rhoades E. Prolactin and cortisol levels following acute alcohol challenges in women with and without a family history of alcoholism. Alcohol 1991;8:383–7.

(33) Ekman AC, Vakkuri O, Vuolteenaho O, Leppaluoto J. Delayed pro-opiopmelanocortin activation after ethanol intake in man. Alcohol Clin Exp Res 1994;18:1226–9.

(34) Inder WJ, Joyce PR, Wells JE, Evans MJ, Ellis MJ, Mattioli L, et al. The acute effects of oral ethanol on the hypothalamic–pituitary–adrenal axis in normal human subjects. Clin Endocrinol (Oxf) 1995;42:65–71.

(35) Gianoulakis C, Krishnan B, Thavundayil J. Enhanced sensitivity of pituitary β-endorphin to ethanol in subjects at high risk of alcoholism. Arch Gen Psychiatry 1996;53:250–7.

(36) Sarkola T, Makisalo H, Fukunaga T, Eriksson CJ. Acute effect of alcohol on estradiol, estrone, progesterone, prolactin, cortisol, and luteinizing hormone in premenopausal women. Alcohol Clin Exp Res 1999;23:976–82.

(37) Rivier C. Alcohol stimulates ACTH secretion in the rat: mechanisms of action and interactions with other stimuli. Alcohol Clin Exp Res 1996;20:240–54.

(38) Longcope C. Dehydroepiandrosterone metabolism. J Endocrinol 1996;150 Suppl:S125–7.

(39) Longcope C. Androgen metabolism and clearance. In: Azziz R, Nestler JE, Dewailly D, editors. Androgen excess disorders in women. Philadelphia (PA): Lippincott-Raven; 1997. p. 37–46.

(40) Longcope C. The endocrinology of the menopause. In: Lobo RA, editor. Treatment of the postmenopausal woman: basic and clinical aspects. 2nd ed. Philadelphia (PA): Lippincott; 1999. p. 35–40.

(41) Hobkirk R. Steroid sulfation. Trends Endocrinol Metab 1993;4:69–74.

(42) Longcope C, Williams KI. The metabolism of estrogens in normal women after pulse injections of ^{3}H-estradiol and ^{3}H-estrone. J Clin Endocrinol Metab 1974;38:602–7.

(43) Ruder HJ, Loriaux L, Lipsett MB. Estrone sulfate: production rate and metabolism in man. J Clin Invest 1972;51:1020–33.

(44) McAllister JM, Hornsby PJ. Dual regulation of 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase, and dehydroepiandrosterone sulfotransferase by adenosine 3′,5′-monophosphate and activators of protein kinase C in cultured human adrenocortical cells. Endocrinology 1988;122:2012–8.

(45) Elekima OT, Mills CO, Ahmad A, Skinner GR, Ramsden DB, Brown J, et al. Reduced hepatic content of dehydroepiandrosterone sulphotransferase in chronic liver disease. Liver 2000;20:45–50.

(46) Henderson BE, Feigelson HS. Hormonal carcinogenesis. Carcinogenesis 2000;21:427–33.

(47) Sasano H, Harada N. Intratumoral aromatase in human breast, endometrial, and ovarian malignancies. Endocr Rev 1998;19:593–607.
(48) Pasqualini JR, Chetrite G. Activity, regulation and expression of sulfatase, sulfotransferase, and 17β-hydroxysteroid dehydrogenase in breast cancer. In: Pasqualini JR, Katznellenbogen BS, editors. Hormone-dependent cancer. New York (NY): Marcel Dekker, Inc; 1996. p. 25–80.
(49) Chetrite GS, Cortes-Prieto J, Philippe JC, Wright F, Pasqualini JR. Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal, and in cancerous, human breast tissues. J Steroid Biochem Mol Biol 2000;72:23–7.
(50) Nasca PC, Liu S, Baptiste MS, Kwon CS, Jacobson H, Metzger BB. Alcohol consumption and breast cancer: estrogen receptor status and histology. Am J Epidemiol 1994;140: 980–8.
(51) Enger SM, Ross RK, Paganini-Hill A, Longnecker MP, Bernstein L. Alcohol consumption and breast cancer oestrogen and progesterone receptor status. Br J Cancer 1999;79:1308–14.
(52) Holm LE, Callmer E, Hjalmar ML, Lidbrink E, Nilsson B, Skoog L. Dietary habits and prognostic factors in breast cancer. J Natl Cancer Inst 1989;81:1218–23.
(53) Potter JD, Cerhan JR, Sellers TA, McGovern PG, Drinkard C, Kushi LR, et al. Progesterone and estrogen receptors and mammary neoplasia in the Iowa Women's Health Study: how many kinds of breast cancer are there? Cancer Epidemiol Biomarkers Prev 1995;4:319–26.
(54) Yoo KY, Tajima K, Miura S, Takeuchi T, Hirose K, Risch H, et al. Breast cancer risk factors according to combined estrogen and progesterone receptor status: a case–control analysis. Am J Epidemiol 1997;146:307–14.
(55) McTiernan A, Thomas DB, Johnson LK, Roseman D. Risk factors for estrogen receptor-rich and estrogen receptor-poor breast cancers. J Natl Cancer Inst 1986;77:849–54.
(56) Normanno N, Ciardiello F, Brandt R, Salomon DS. Epidermal growth factor-related peptides in the pathogenesis of human breast cancer. Breast Cancer Res Treat 1994;29:11–27.
(57) Dickson RB, Johnson MD, Bano M, Shi E, Kurebayashi J, Ziff B, et al. Growth factors in breast cancer: mitogenesis to transformation. J Steroid Biochem Mol Biol 1992;43:69–78.
(58) Manni A. The role of polyamines in the hormonal control of breast cancer cell proliferation. Cancer Treat Res 1994;71:209–25.
(59) Liehr JG, Ricci MJ. 4-Hydroxylation of estrogens as markers of human mammary tumors. Proc Natl Acad Sci U S A 1996;93:3294–6.
(60) Cavalieri EL, Stack DE, Devanesan PD, Todorovic R, Dwivedy I, Higginbotham S, et al. Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. Proc Natl Acad Sci U S A 1997;94: 10937–42.
(61) Altman DG. Practical statistics for medical research. London (U.K.): Chapman & Hall; 1991. p. 210–1.

NOTES

Editor's note: D. W. Chandler owns stock in Esoterix, Inc., which owns the laboratory where some of the sample testing was performed.

Manuscript received October 11, 2000; revised February 21, 2001; accepted March 6, 2001.